State of New Hampshire Department of Safety
Division of State Police
Forensic Laboratory

Protocol Title	Identification of Gamma-Hydroxybutyric Acid (GHB), 1,4-Butanediol (1,4-BD), and Gamma-Hydroxybutyrolactone (GBL)		
Method Title	Analysis Specifics		
Reviewed and Approved By (signature/date)	**UNCONTROLLED COPY**		
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Effective Date:	01/04/10	Date Due for Revision:	12/31/11

Reviewed and Re-App. By		Next Due for Revision:
Re-Authorized By		12/31/13

Reviewed and Re-App. By		Next Due for Revision:
Re-Authorized By		12/31/15

OBJECTIVE

To determine if a sample of evidence contains Gamma-Hydroxybutyric Acid (GHB), a controlled drug, or 1,4-Butanediol (1,4-BD) and Gamma-Hydroxybutyrolactone (GBL), analogues of the controlled drug GHB

SCOPE

This analysis applies to any sample that may have been subjected to any or all of the methods in the protocol for Identification of Drugs (DRU-001) and is suspected at some point to be or contain GHB, 1,4-BD, or GBL. Any analyst who is properly trained for this method may perform this analysis.

REFERENCES

Morris, Jeremiah A., *Extraction of GHB for FTIR Analysis and A New Color Test for Gamma-Butyrolactone (GBL)*, Microgram, Vol. XXXII, No. 8, August, 1999, pp. 215-221

PRECAUTIONS (DRU-304)

- Hotplate (section 6)
- The analyst must be familiar with precautions noted in the MSDS for each reference material.
- **Acetonitrile is a possible cyanide poison with delayed effects that are life threatening.** This solvent is extremely flammable; caution should be exercised when working with equipment at high temperatures. This solvent is a possible mutagen, teratogen and toxic when in contact with skin or inhaled. It shall be worked with in a hood and gloves shall be worn. Gloves act as a barrier, but they offer minimal protection. If not wearing thick gloves and contact with these chemicals occur, immediately remove gloves and wash the affected area thoroughly.
- Hazardous chemicals (section 5):

Potassium bromide (KBr)	Ethyl ether
Chloroform	Sulfuric acid
Sodium sulfate	Celite
Drug standards	

SUPPLIES, REAGENTS and EQUIPMENT

sulfuric acid (GEN-101)

acetonitrile (J.T.Baker #2-9011 or equivalent)
ethyl ether (EM Science #EX0194-7 or equivalent)
chloroform (Mallinckrodt #4441 or equivalent)
potassium bromide (KBr) (Mallinckrodt #0500-02 or equivalent)
sodium sulfate (Mallinckrodt #8024 or equivalent)
Celite (Manville Corporation or equivalent)
pH paper (pHydrion, pH 1-11 or equivalent)
Filter paper (Whatman #L005-055 or equivalent)
12 x 75 mm test tubes (Kimax 51 borosilicate or equivalent)
shell vial (12 x 35 mm, Kimbal #60931-2)
various glassware
hotplate
calibrated balance (GEN-201)
thermometer 0 to 100°C (approximate range or equalivalent)
UV-VIS (HP 8453 or equivalent)
FTIR (Nicolet Magna 560, Nicolet 380 or equivalent)
GC/MS (Varian Saturn 2000 series, Thermo GC/DSQ or equivalent)
BSTFA [N, O-bis (trimethylsilyl) trifluoracetamide] (Pierce #38830 or equivalent)
1, 4-Butanediol [1, 4-BD] (Aldrich #24,055-9 or equivalent)
gamma-Hydroxybutyrolactone [GBL] (Sigma #H-7629 or equivalent)
gamma-Hydroxybutyric Acid Sodium Salt [GHB] (Sigma #H-3635 or equivalent)

PROCEDURE

1. Report all observations on attachment I to DRU-001 and if necessary attachment II.

2. If a sample of material is suspected to contain GHB, 1-4-Butanediol, or GBL there is a general procedure of analysis to be followed per DRU-001.

3. More specifically, the analysis of GHB, 1,4-BD, and GBL involves the following:
   - 3.1. Macroscopic examination
     - 3.1.1. Determine the net weight.
     - 3.1.2. Determine sample volume (optional).
     - 3.1.3. Annotate the appearance of the material in regards to color, odor and consistency.
     - 3.1.4. Determine the pH of the material if the sample is a liquid.
     - 3.1.5. (Optional) Determine if the liquid is aqueous in nature
       - ❑ Aqueous samples are miscible with water and immiscible with $CHCl_3$.
   - 3.2. (Optional) Direct FTIR analysis
     - 3.2.1. Liquid samples may be run direct using the ATR accessory and a cover slip.
     - 3.2.2. Aqueous samples may be dried to a residue and then run on the FTIR.
     - 3.2.3. Pure or concentrated samples with little or no interfering compounds may yield an FTIR spectrum which is at least indicative for one of the above drugs.
       - ❑ Samples in sugar-based beverages do not usually yield direct spectra of any value. An extraction is recommended for this type of sample before instrumental analysis.
   - 3.3. Recommended Color Tests
     - 3.3.1. For GHB: GHB Color Test (DRU-121)
     - 3.3.2. For GBL: Cobalt Thiocyanate (DRU-102)

- Pure GBL will turn an immediate bright blue when a drop is placed in a well containing cobalt thiocyanate reagent.
  - ✓ Diluted samples may need to be extracted to yield positive results with this test.

3.4. UV/VIS Analysis (DRU-200)

3.4.1. No reported absorbance in 0.1 N H_2SO_4 for the three materials in question.

3.4.2. Presence of activity indicates that other materials may be present.

3.5. TLC analysis (Refer to Thin Layer Chromatography Test Procedures, DRU-306)

3.5.1. The general TLC system (Ethyl Acetate : Methanol : NH_4OH, 17 : 2 : 1) will separate and distinguish GBL and 1,4 BD. GHB, due to its insolubility in common organic solvents, remains at the origin in this system.

3.6. Microcrystal (Refer to Microcrystal Test Procedures, DRU-301)

3.6.1. None currently in use.

3.7. Extractions (may be necessary prior to instrumental analysis):

3.7.1. GBL and 1,4 BD are viscous liquids in their pure state. They are soluble in water, alcohol and $CHCl_3$. GHB salt is a white powder in its pure state. It is soluble in water and insoluble in $CHCl_3$. These solubility characteristics can be helpful in extracting the drugs from various substrates.

3.7.2. Liquid Samples (Volumes may be proportionately adjusted and other appropriate glassware used if necessary).

- If GHB was indicated in the sample screening tests and the solution appears to be water-based:
  - ✓ Using a separatory funnel wash approximately 10 ml of the sample solution at least twice with 10 ml aliquots of chloroform to remove impurities.
  - ✓ Dry the remaining aqueous fraction and perform an FTIR analysis (see 3.9).
  - ✓ If the spectrum is still only indicative for GHB, place the dried residue in filter paper and wash it again with "dried" chloroform (chloroform which has been passed through sodium sulfate).
  - ✓ Repeat the FTIR on the dried residue.
  - ✓ If the resultant spectrum is still not satisfactory, the sample will need to be derivatized and run on the GC/MS (see 3.8).
- If GBL or 1,4 BD was indicated in the sample screening tests:
  - ✓ Using a separatory funnel, perform two 10 ml chloroform extractions of approximately 10 ml of the sample solution.
  - ✓ Run the chloroform extracts through sodium sulfate to remove excess water.
  - ✓ Evaporate the chloroform extract and analyze the resultant residue using FTIR (see 3.9) or GC/MS (see 3.8).
- If initial screening tests failed to indicate either GHB, GBL or 1,4 BD:
  - ✓ Place 10 ml of the solution in a separatory funnel. Extract the sample twice with 10 ml of chloroform.
  - ✓ Pass the chloroform extract through sodium sulfate and evaporate to dryness.

- ✓ Also evaporate the remaining aqueous solution to dryness.
- ✓ Perform FTIR analysis (see 3.9) on any resulting residues in the two above extractions. If necessary also perform GC/MS testing (see 3.8).

- Alternately, ethyl ether can be used to wash/extract samples.
  - ✓ GHB acid form and 1,4 BD are soluble in ether; GHB salt form is insoluble in ether and will remain in the aqueous fraction.
  - ✓ Run ethyl ether extracts through sodium sulfate to remove excess water prior to evaporation.
  - ✓ Evaporate ether/aqueous extracts and perform FTIR analysis (see 3.9) and/or GC/MS analysis (see 3.8) on any residues obtained.

3.7.3. Solid, powders or dried residues

- If GHB was indicated in the sample screening tests:
  - ✓ Suspend or dissolve a portion of the material in approximately 10 ml of 0.1N H_2SO_4.
  - ✓ Using a separatory funnel, wash the sample solution at least twice with 10 ml aliquots of chloroform to remove impurities.
  - ✓ Dry the remaining aqueous fraction and perform an FTIR analysis (see 3.9).
  - ✓ If the spectrum is still only indicative for GHB, place the dried residue in filter paper and wash it again with "dried" chloroform (chloroform which has been passed through sodium sulfate).
  - ✓ Repeat the FTIR on the dried residue.
  - ✓ If the resultant spectrum is still not satisfactory, the sample will need to be derivatized and run on the GC/MS (see 3.8).
- If GBL or 1,4 BD was indicated in the sample screening tests:
  - ✓ Suspend or dissolve a portion of the material in approximately 10 ml of 0.1N H_2SO_4.
  - ✓ Using a separatory funnel, extract the sample solution twice with 10 ml aliquots of chloroform.
  - ✓ Pass the chloroform extracts through sodium sulfate to remove any moisture.
  - ✓ Evaporate the chloroform extract and analyze the resultant residue using FTIR (see 3.9) or GC/MS (see 3.8).

3.8. GC/MS (DRU-202 or DRU-209)

3.8.1. Sample is run direct if relatively pure or extracted in an appropriate solvent (see above).

3.8.2. Derivatization Method for samples containing GHB

- Without derivatization, GHB will convert to GBL in the inlet and will be identified as such on the mass spectrometer.
- To run GHB, as a derivatized material, add a small portion of dried sample, no more than 10 milligrams is required, to a micro reaction vessel.
- Add 50 ul of derivatizing reagent – BSTFA and cap the vial.
- Allow the mixture to stand for at least one half hour to complete the reaction.
- Add 100 ul of dried acetonitrile (acetonitrile passed through anhydrous sodium sulfate) to the sample and reagent mixture.
- Cap the micro reaction vessel and heat the solution to approximately 45°C for at least 10 minutes.
- Dilute the resulting product with chloroform to the ratio of 1 part sample to 3

parts chloroform.
- Run the sample on the on GC/MS using the GHB program.
- Product ions: molecular ion - 233
- principal ions – 147, 73,75, 117, 148, 204, 133

3.8.3. Underivatized GC/MS Run for samples that are suspected to contain GBL or 1, 4-BD on any instrument and any inlet.
- Both GBL and 1, 4-Butanediol are soluble in chloroform.
- Perform a chloroform extraction of the suspect solution.
- The extract can be dried to a useable volume and run on the GC/MS using a GHB run program.
- GBL has a molecular ion of 87. The remaining principal peaks are 41, 42, 39, 40 and 56.
- 1, 4-BD has a molecular ion of 91. The remaining principal peaks are 43, 41, 42, 44, 71, 57 and 73.

3.8.4. The sample mass spectrum is compared to mass spectra of known reference materials. (Refer to DRU-001-01 for interpretation guidelines.)

3.8.5. If GC retention time is to be used as a presumptive test, the RT value must be compared with a contemporaneously run reference material (DRU-001-01).

3.9. FTIR (GEN-211)

3.9.1. Analysis can be performed utilizing KBr method or ATR accessory for sample or sample extraction.

3.9.2. Sample spectrum is compared to spectra from known reference materials. (Refer to DRU-001-01 for interpretation guidelines).

3.10. RAMAN (GEN-212)

3.10.1. It is recommended that this testing be used only with extracted samples. Liquid samples in which the concentration of GHB is 5% or greater will provide favorable spectral matching for GHB.

3.10.2. Compare sample spectrum with spectra from known reference materials (refer to DRU-001-01 for interpretation guidelines).

CONCLUSIONS

1. If sufficient sample is available, the analysis has identified the presence of GHB if the following two criteria (1.1 and 1.2) are met:
   - 1.1. The sample exhibits at least one of the following:
     - 1.1.1. Positive results for the GHB color test
     - 1.1.2. A FTIR or RAMAN spectrum that is at least indicative for GHB.
   - 1.2. A sample of evidence exhibits one (1) of the following:
     - 1.2.1. A mass spectrum that matches a derivatized GHB reference material
     - 1.2.2. A FTIR or RAMAN spectrum that matches a GHB reference material (If option 1.1.2 was used above, the confirmatory instrument must be different from the one used in the presumptive testing, e.g. FTIR presumptive = RAMAN confirmatory and vice-versa).

2. If sufficient sample is available, the analysis has identified the presence of GBL or 1,4-BD if both of the following criteria (2.1 and 2.2) are met:
   - 2.1. The sample must exhibit at least one of the following:
     - 2.1.1. An FTIR or RAMAN spectrum that is at least indicative for GBL or 1,4-BD

2.1.2. A positive cobalt thiocyanate color test for GBL

2.2. One of the following conditions must be met:

2.2.1. The sample exhibits a mass spectrum that matches an appropriate reference material

2.2.2. The sample exhibits a FTIR or RAMAN spectrum that matches an appropriate reference material. (If option 2.1.1 was used above, the confirmatory instrument must be different from the one used in the presumptive testing, e.g. FTIR presumptive = RAMAN confirmatory and vice-versa).

3. With limited sample the analysis has identified the presence of GHB, GBL or 1, 4-BD if both of the following criteria are met:

3.1. A GC retention time matches an appropriate reference material

3.2. A mass spectrum matches an appropriate reference material. (In the case of GHB, a derivatized spectrum). (The MS must be run on a different instrument than the one used to determine the RT).

4. For exhibits containing multiple similar items with suspected GHB, GBL or 1,4 BD:

4.1. Follow multiple sampling procedure DRU-001-02.

DISPOSAL (SA-090)

1. Organic ethyl ether and chloroform wash waste = **OA**
2. Aqueous fractions = **OA**